Renal and Segmental Artery Hemodynamics During Whole-Body Passive Heating and Cooling Recovery

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Abstract

High environmental temperatures are associated with increased risk of acute kidney injury, which may be related to reductions in renal blood flow. The susceptibility of the kidneys may be increased due to heat stress-induced changes in renal vascular resistance to sympathetic activation. We tested the hypotheses that, compared to a normothermia, increases in renal vascular resistance (RVR) during the cold pressor test (CPT, a sympathoexcitatory maneuver) are attenuated during passive heating, and exacerbated following cooling recovery. Twenty-four healthy adults (22 ± 2 years, 12 females) completed CPTs at normothermic baseline, after passive heating to a rise in core temperature of ~1.2°C, and following cooling recovery when core temperature returned to ~0.2°C above normothermic baseline. Blood velocity was measured using Doppler ultrasound in the distal segment of the right renal artery (Renal, n=24 during thermal stress, n=12 during CPTs) or middle portion of a segmental artery (Segmental, n=12). RVR was calculated as mean arterial pressure divided by renal or segmental blood velocity. RVR increased at the end of CPT during normothermic baseline in both arteries (Renal: by 1.0 ± 1.0 mmHg/cm/s, Segmental: by 2.2 ± 1.2 mmHg/cm/s, $P \leq 0.03$), and these increases were abolished with passive heating ($P \geq 0.76$). At the end of cooling recovery, RVR in both arteries to the CPT was restored to that of normothermic baseline ($P \leq 0.17$). These data show that increases in RVR to sympathetic activation during passive heating are attenuated and return to that of normothermic baseline following cooling recovery.

Keywords: renal vascular resistance, renal blood velocity, cold pressor test, Doppler ultrasound, heat stress, whole-body cooling, sympathetic activation

New & Noteworthy:

Our data indicate that increases in renal vascular resistance to the cold pressor test (i.e., sympathetic activation) are attenuated during passive heating, but at the end of cooling recovery this response returns to that of normothermic

baseline. Importantly, hemodynamic responses were assessed in arteries going to (renal artery) and within (segmental artery) the kidney, which has not been previously examined in the same study during thermal and/or sympathetic stressors.

INTRODUCTION

The risk of hospital admissions from acute kidney injury increases by ~23% per 1°C increase in ambient temperatures above ~29°C (31). The etiology underlying this kidney injury is largely unexplored. Heat stress elicits reductions in renal blood flow in humans (1, 6, 22, 29, 30, 36, 37, 41, 44), and subsequently, increases the risk of localized renal ischemia (27, 38). Elevations in body temperature and the associated loss of body fluid due to sweating, elevate renal oxygen demand subsequent to fluid and sodium reabsorption (11). This likely increases the susceptibility of the kidneys to injury during heat stress (16), particularly during this relatively low blood flow state. Thus, there is a need to understand the mechanisms and modulators of renal hemodynamics during heat stress.

Changes in renal vascular control can be probed using sympathoexcitatory stimuli (e.g., cold pressor test, face cooling, handgrip exercise) to examine differences in renal vascular resistance to general sympathetic stimulation (13, 39, 40, 47). The effect of heat stress on changes in renal vascular resistance to sympathetic stimulation compared to normothermia is currently unclear. There is some evidence of a heightened increase in renal vascular resistance during non-exercise sympathetic activation (i.e., head up tilt) during mild passive heat stress (~0.4°C increase in esophageal temperature) (37). By contrast, a re-examination of data from Smith et al. suggests that the exercise-induced increase in renal vascular resistance is attenuated during heat stress (52). Notably, these findings align with previous data indicating an attenuated increase in vascular resistance to non-exercise sympathetic activation in peripheral vascular beds during heat stress compared to normothermia (10, 49). Nevertheless, if heat stress modifies non-exercise sympathetically stimulated changes in renal vascular resistance warrants further investigation.

Rapid whole-body cooling is known to alleviate many health-risks associated with heat stress and subsequent elevations in core body temperature (e.g., heat stroke) (7, 18, 20). It is conceivable that cooling following passive heat stress may neutralize decrements in renal blood flow, thereby protecting the kidneys. However, in the absence of prior heat stress, an ~2°C reduction in mean skin temperature increases renal vascular resistance by ~12% (56). Thus, there is the potential that active cooling immediately following heat stress could result in exacerbated reductions in renal perfusion. However, this remains unknown.

Renal vascular responses during heat stress have traditionally been studied using sodium para-aminohippuric acid (PAH) clearance to measure renal plasma flow (25, 37, 44, 52). However, this technique may not be appropriate for measuring renal vascular control during brief periods of sympathoexcitation (e.g., 2 min cold pressor test) because of the rapid changes in renal blood flow during these short protocols that depend on a steady state of PAH infusion/excretion. Doppler ultrasound is commonly used in both clinical and research settings to estimate acute changes in renal vascular control (40, 43, 47, 56). In this context, the purpose of this study was to examine the acute changes in renal vascular resistance to the cold pressor test (i.e., a sympathoexcitatory stimulus) during passive heating and following cooling recovery using Doppler ultrasound. The cold pressor test was employed because increases in muscle sympathetic nerve activity (i.e., an indicator of general sympathetic activity) during the cold pressor test do not differ between normothermic and heat stress conditions (10). We hypothesized that increases in renal vascular resistance during the cold pressor test would be attenuated during passive heating compared to normothermic baseline. Additionally, we hypothesized that this increase in renal vascular resistance would be exacerbated following cooling recovery from prior heat stress compared to normothermic baseline.

METHODS

Participants

Twenty-four healthy adults (12 females) participated in this study. Participant characteristics were – age: 22 ± 2 years, height: 170 ± 9 cm, and weight: 67 ± 12 kg. Participants provided written consent after being fully informed of the experimental procedures and possible risks. Participants reported to be free from any known cardiovascular, metabolic, renal, or neurological diseases, and were physically active nonsmokers. Female participants were not pregnant, which was confirmed via a urine pregnancy test, and self-reported to be normally menstruating. Females were tested during the first ten days of their self-identified menstrual cycle. This study was approved by the Institutional Review Board at the University at Buffalo and performed in accordance with the standards set by the latest revision of the Declaration of Helsinki.

Instrumentation and Measurements

Nude body weight and height were measured using a stadiometer and scale (Sartorius Corp., Bohemia, NY, USA). Urine specific gravity was measured in duplicate using a refractometer (Atago USA, Inc., Bellevue, WA, USA).

Core temperature was measured using an ingestible telemetry capsule (HQ Inc., Palmetto, FL, USA). Thermocouples (Omega Engineering, Inc. Stamford, CT, USA) were used to continually measure mean skin temperature using the weighted average of six locations as previously described (49). Body temperature was controlled using a tube-lined water-perfused suit (Med-Eng, Ottawa, ON, Canada) which covered the entire body except for the head, hands, and feet. The water-perfused suit top for the upper body was affixed together using clamps in lieu of the zipper, which allowed us to keep the anterior portion of the body, to just inferior to the level of the xyphoid process, in contact with the water perfused-suit during Doppler ultrasound measurements. During these measurements, the clamps on the lower portion of the suit top were removed, and the right side of the suit was folded back enough to expose lateral aspect of the lower right ribs (i.e., the ultrasound measurement location). Heart rate was continually measured via a 3-lead ECG (DA100C, Biopac Systems, Inc., Goleta, CA, USA). Beat-to-beat blood pressure was measured via the Penaz method (Finometer Pro, FMS, Amsterdam, Netherlands), which was intermittently confirmed via auscultation of the brachial artery by electrosphygmomanometry (Tango M2, SunTech, Raleigh, NC, USA). Blood pressure data from the Penaz method are reported.

Renal blood velocity was measured in the distal segment of the right renal artery (renal artery) and in the middle portion of the same segmental artery in the right kidney (segmental artery) for that participant. Renal and segmental artery blood velocities were obtained via Doppler ultrasound (GE Vivid 7 Dimension, Chicago, IL, USA). Due to the effects of respiration on moving the renal and segmental arteries during ultrasound measurements (40), participants were instructed to perform a mid-exhalation, non-Valsalva breath hold for no longer than 10 seconds. Importantly, mean renal blood velocity measured after breath holding up to 40 seconds has previously been shown to not differ from measurements taken during various breathing frequencies (53). Given these circumstances, we determined that utilizing a short breath hold duration was optimal in order to minimize variation caused by moving of the arteries during respiration. The coronal approach was utilized (47), which allowed us to delineate the renal artery as it enters the kidney and observe distinct branching into segmental arteries in the renal pelvis. With participants in the left lateral recumbent position, a phased-array transducer (2.5-3.5 MHz) was held in the same location for all measurements after marking the transducer location, which was marked with indelible ink during baseline measurements. The focal zone was set to the artery's depth and the insonation angle was <60°. Mean renal and segmental artery blood velocities were indexed from the waveform envelope by the time-averaged maximum velocity. Mean blood velocity was measured and averaged over three cardiac cycles as has been done previously (40, 47) due to the practical limitations associated with the relatively small window to obtain

measurements during the cold pressor test. All renal measurements were obtained by the same sonographer (CLC) and were extracted by a separate member of the investigative team (JMB). The transducer was removed from the body during periods of heating and cooling (i.e., non-cold pressor test measurement periods). Thus, optimization and measurement of blood velocity in the renal and segmental arteries occurred within 2 min of when the transducer was replaced on the participant during these heating and cooling periods. Importantly, using this approach, the within-subject test-retest coefficient of variation for blood velocity measurements were 3.9 ± 0.8% (renal artery) and 3.9 ± 1.2% (segmental artery). Further, the transducer held in place throughout the duration of the cold pressor tests (i.e., it was not removed from the body), and image acquisition occurred within a 10 second window. Given the depth of the renal and segmental artery, it is not possible to accurately measure vessel wall diameter. However, renal blood velocity was interpreted to reflect changes in renal blood flow as has been done previously (13, 14, 39, 40, 43, 47, 56) because during pharmacologically induced renal vasoconstriction, changes in renal artery blood flow were due to changes in blood velocity and not diameter (33).

Experimental Protocol

Overview

During one experimental visit, participants completed a cold pressor test (CPT, described below) at normothermic baseline, following passive heating, and following a passive cooling recovery to examine acute changes in the renal vascular resistance to sympathetic activation during these changes in body temperature (**Figure 1**). This experiment was initially performed in 12 participants only measuring renal artery blood velocity. However, after finding robust responses of the renal artery to the cold pressor test, and a surprising finding of a lack of increased vascular resistance in the renal artery to passive heating, we recruited an additional 12 participants to investigate if similar changes occurred in the segmental artery. In these participants, we measured both the renal and segmental arteries during the heating and cooling periods, and only measured blood velocity in the segmental artery during the cold pressor test. Thus, during the heating and cooling periods, we had 24 participants for renal artery measurements and 12 for segmental artery. Additionally, we had two separate cohorts of 12 participants each for renal and segmental artery measurements during the cold pressor tests.

Normothermic baseline

Participants reported to the temperature-controlled laboratory (25 ± 1°C, 25 ± 11% relative humidity) after abstaining from exercise, caffeine, and alcohol for 12 h, and food for 3 h. To control for diurnal changes in renal function,

all participants arrived at the laboratory within the same two-hour window (12:00pm – 2:00pm). Euhydration was confirmed upon arrival via a urine specific gravity <1.020. Participants consumed an ingestible telemetry capsule for measurement of core temperature, and then were weighed nude in a private room. Participants were not allowed to drink any fluids thereafter. Participants were then instrumented as described above. Following instrumentation, participants assumed the supine position for 10 min of quiet rest with the temperature of the water-perfused suit controlled to 34°C. Baseline measurements were taken over the next 5 min (normothermic baseline). At the end of this period, the CPT was administered for 2 min by submerging the participants right hand into an agitated ice slurry mixture up to the wrist. The CPT is a sympathetic maneuver which stimulates the nociceptors and subsequently increases vascular resistance and blood pressure (10). Renal blood velocity data were collected at baseline (Pre-CPT), at 1 and end of the CPT (1 min and End), and 1 and 5 min into the recovery period following the CPT (Post-CPT).

Passive heating

Following CPT measurements, passive heat stress commenced. Passive heating was induced by perfusing the suit with 50°C water. To impede evaporative heat loss and promote heat gain, participants were wrapped in a Mylar blanket (Primacare Medical Supplies, Passaie, NJ, USA) with standard commercial bath towels placed over the blanket. Renal blood velocity measurements were taken when core temperature increased 0.6 ± 0.1°C above normothermic baseline, and passive heating continued until participant core temperature increased to 1.2 ± 0.1°C. At this point the mylar blanket and towels were removed, and the temperature of the water perfusing the suit was reduced to 45°C to attenuate the continued increase in core temperature. Then, a CPT was administered and measurements were taken as described during normothermic baseline.

Passive cooling recovery

Following the final measurements of the passive heating CPT, the temperature of the water-perfused suit was switched to 20°C to promote the recovery of core temperature towards normothermic baseline. Renal blood velocity data were collected at the time mean skin temperature returned to normothermic baseline (within -0.1 ± 0.4°C) but core temperature remained elevated (by 1.2 ± 0.3°C), and when core temperature returned to 0.6 ± 0.1°C above normothermic baseline. Cooling continued until core temperature returned to normothermic baseline or plateaued for >5 min (by 0.2 ± 0.2°C). Then, the final CPT was administered and measurements were taken as described above. Participants were then de-instrumented and a final nude body weight was taken.

Data and Statistical Analyses

Renal (n=24) and segmental (n=12) artery measurements were taken at all stages of baseline, passive heating, and cooling recovery. Renal blood velocity measurements were taken in separate cohorts (renal artery [n=12] and segmental artery [n=12]) during the CPT. Both renal and segmental artery measurements during the CPT were not feasible due to the time constraints imposed by the 2 min duration of the CPT. Adequate ultrasound images were obtained in 96.9% of renal artery measurements (279 out of the possible 288, with 7 missing due to acoustic shadowing of the kidney and 2 missing due to technical difficulties) and 96.8% of segmental artery measurements (209 out of 216, with 4 missing due to acoustic shadowing of the kidney and 3 missing due to technical difficulties). All non-ultrasound data were sampled continuously at 1000 Hz via a data acquisition system (Biopac MP150, Goleta, CA, USA). Heart rate and mean arterial pressure were extracted during the same three cardiac cycles for all renal measurements described above. Renal vascular resistance was calculated as [mean arterial pressure / renal blood velocity].

All data were analyzed using Prism software (Version 8, GraphPad Software Inc., La Jolla, CA, USA). Data were analyzed as absolute values during baseline, passive heating and cooling recovery using one-way ANOVA. During the CPT, data were analyzed as the absolute change from Pre-CPT using two-way ANOVA. The CPT data were analyzed as the absolute change to examine the responses to the CPT independent from differences that we expected pre-CPT. Absolute data at Pre-CPT were analyzed using one-way ANOVA. A mixed-effects model ANOVA was used to account for any missing data points (described above) in the ultrasound-based data. When an ANOVA revealed a significant main effect for time, condition (i.e., Normothermia, Heated, and Cooling Recovery), or a significant interaction, post hoc Holm-Sidak test pairwise comparisons were made. Comparisons between the absolute change from Pre-CPT in renal blood velocity and renal vascular resistance in the renal and segmental arteries were made using unpaired t-tests for a given condition. A priori statistical significance was set at $P < 0.05$, and actual P-values are reported where possible. Data are reported as mean ± SD.

RESULTS

Participants lost 1.2 ± 0.5% of body weight throughout the trial.

Responses to Passive Heating and Cooling Recovery

Participants were passively heated to an increase in core temperature of 1.2 ± 0.1°C ($P < 0.01$, **Table 1**). At the end of passive heating mean arterial pressure decreased by 10 ± 7 mmHg compared to normothermic baseline ($P < 0.01$,

Figure 2A). Blood velocity in the renal artery decreased at the end of passive heating by -6.0 ± 4.4 cm/s ($P < 0.01$, **Figure 2B**), but did not differ from baseline in the segmental artery ($P = 0.10$, **Figure 2E**). At the end of passive heating, vascular resistance was not different from normothermic baseline in the renal ($P = 0.54$, **Figure 2C**) and segmental arteries ($P = 0.99$, **Figure 2F**).

The renal vascular response to cooling recovery was measured at the exact time mean skin temperature returned to normothermic baseline, but core temperature remained elevated (by 1.2 ± 0.3°C, $P < 0.01$, **Table 1**). During this period, mean arterial pressure remained reduced below normothermic baseline (by -8 ± 10 mmHg, $P < 0.01$, **Figure 2A**). Blood velocity in the renal artery returned to normothermic baseline ($P = 0.61$), and was significantly increased compared to the end of passive heating (by 4.6 ± 5.3 cm/s, $P < 0.01$, **Figure 2C**). In the segmental artery, blood velocity did not differ from baseline or passive heating ($P \geq 0.73$, **Figure 2F**). Lastly, at this time point, vascular resistance did not differ from passive heating and normothermic baseline in both renal and segmental arteries ($P \geq 0.22$, **Figure 2C, F**). Cooling recovery continued until core temperature returned to 0.2 ± 0.2°C above baseline levels (**Table 1**). At this period, mean arterial pressure was increased by 11 ± 7 mmHg compared to normothermic baseline ($P < 0.01$, **Figure 2A**). Blood velocity was increased above passive heating in the renal (by 3.5 ± 4.7 cm/s, $P < 0.03$, **Figure 2B**) and segmental (by 4.0 ± 4.0 cm/s, $P < 0.05$, **Figure 2E**) arteries, but was not different from normothermic baseline in either ($P \geq 0.14$). The end of cooling recovery resulted in an elevated vascular resistance in the renal artery compared to normothermic baseline, end of passive heating, and the initial cooling period when skin temperature returned to normothermic baseline ($P < 0.04$, **Figure 2C**). There were no statistical differences in vascular resistance at the end of cooling recovery in the segmental artery between any time points ($P \geq 0.32$, **Figure 2F**).

Normothermic renal response to the cold pressor test

Pre-CPT values during normothermic baseline, end of passive heating, and end of cooling recovery are reported in **Table 1 and Figure 2**. Blood velocity was reduced in the segmental artery (by -5.2 ± 3.5 cm/s, $P < 0.01$, **Figure 3E**), but not the renal artery ($P = 0.85$, **Figure 3B**), during the baseline CPT. These reductions in segmental artery blood velocity were resolved at 1 min Post-CPT (**Figure 3E**). At the end of the CPT, vascular resistance increased from Pre-CPT in the renal and segmental arteries (Renal: by 1.0 ± 1.0 mmHg/cm/s, Segmental: by 2.2 ± 1.2 mmHg/cm/s, $P \leq 0.03$) and returned to Pre-CPT levels at 1 min Post-CPT in both arteries ($P \geq 0.13$, **Figure 3F**).

Renal response to the cold pressor test following passive heating and cooling recovery

Following passive heating, increases in mean arterial pressure at the end of CPT were attenuated compared to normothermic baseline (3 ± 5 vs. 25 ± 11 mmHg, $P < 0.01$, **Figure 3**). Additionally, there were no changes from Pre-CPT in blood velocity or vascular resistance in the renal or segmental arteries during or following the CPT after passive heating ($P \geq 0.15$, **Figure 3).** In the renal artery, vascular resistance at 1 min Post-CPT was lower with passive heating compared to normothermic baseline (-0.3 ± 0.3 vs. 0.2 ± 0.3 mmHg/cm/s, $P < 0.01$, **Figure 3C**).

Cooling recovery restored the mean arterial pressure response to the CPT (**Figure 3**). Blood velocity in the renal artery was lower in cooling recovery compared to passive heating at End (-3.5 ± 5.0 vs. 2.2 ± 6.2 cm/s, $P = 0.06$) and 1 min Post-CPT (-2.7 ± 4.9 vs. 3.3 ± 5.4 cm/s, $P = 0.02$, **Figure 3B**). However, there were no differences in blood velocity in the renal or segmental arteries from Pre-CPT or compared to normothermic baseline and passive heating at any other time point following cooling recovery ($P \geq 0.20$, **Figure 3**). The renal vascular resistance response to the CPT was restored to normothermic baseline following cooling recovery. Vascular resistance in the renal and segmental arteries was elevated above passive heating at End and 1 min-Post CPT ($P \leq 0.04$) but did not differ from normothermic baseline throughout ($P \geq 0.17$, **Figure 3**).

Comparison of renal and segmental artery response to the cold pressor test

There were no differences in blood velocity between renal and segmental arteries at the end of the CPT ($P \geq 0.16$, **Figure 4A**). The increase in vascular resistance was greater in the segmental artery than renal artery at normothermic baseline (2.2 ± 1.2 vs. 1.0 ± 1.0 mmHg/cm/s, $P < 0.02$, **Figure 4B**). These differences were no longer detected at the end of CPT following passive heating and cooling recovery ($P \geq 0.21$, **Figure 4B**).

DISCUSSION

In support of our hypothesis, increases in renal vascular resistance to sympathetic activation are attenuated during passive heating. Contrary to our second hypothesis, our data indicate that the increases in renal vascular resistance to sympathetic activation are not exacerbated at the end of cooling recovery following passive heating. These renal vascular responses to passive heating and cooling recovery were detected in both blood flow to the kidney (renal artery) and blood flow within the kidney (segmental artery). To our knowledge, this is the first study to measure renal hemodynamics in the renal and segmental artery with Doppler ultrasound during passive heating, cooling recovery, and during sympathetic activation. Importantly, these data provide insight into changes in renal hemodynamics during thermal stress accompanied by a sympathoexcitatory maneuver.

Renal hemodynamics during passive heating and sympathetic activation

High ambient temperatures (≥42°C) decrease renal blood flow by ~20-40% in resting humans, the magnitude of which is dependent on the length of exposure and/or relative humidity of the environment (1, 22, 29, 30, 41, 44). Similar decreases in renal blood flow have been reported across a wide range of increases in core temperature (by ~0.4-1.9°C above baseline) using a water-perfused suit (36, 37, 46). Using PAH clearance to estimate renal blood flow, both models demonstrate that an increase in renal vascular resistance (by ~16-32%) is the primary driver for reductions in renal blood flow (36, 37, 41). These data are supported by studies in baboons demonstrating that increases in renal vascular resistance induced by heat stress are mediated by the sympathetic nervous system (15). In humans, these increases in renal vascular resistance occurring during supine passive heating are often interpreted as occurring in support of the large redistribution of blood flow towards the skin to promote heat dissipation (36, 45).

After passive heating in the present study, we did not detect increases in vascular resistance in the renal and segmental arteries. Rather, the 16% reduction in renal artery blood velocity was likely a consequence of reductions in perfusion pressure, as indexed by decreases in mean arterial pressure. Previous work in humans has used PAH clearance to estimate decreases in renal blood flow during heat stress occurring at the level of the nephron (i.e., filtration). This is in contrast to our approach with Doppler ultrasound, where we are able to estimate blood flow to (renal artery) and within (segmental artery) the kidneys at locations upstream to the nephron. It is possible that vascular resistance in the renal and segmental arteries increased with passive heating, but that we were unable to detect these changes in renal vasomotor tone due to an inability to measure artery diameter using Doppler ultrasound. We think this is unlikely given that the diameter of the renal artery does not change during pharmacologically induced renal vasoconstriction (33). Thus, the reason for the differential findings between our data and those using PAH clearance is unclear. It is notable, however, that the PAH clearance techniques provide an index of renal blood flow downstream to the location of measurement we used with Doppler ultrasound. We speculate that these conflicting findings between our data and those using PAH clearance lend support to a heterogenous redistribution of intrarenal blood flow (i.e., non-uniform changes in renal cortical and medullary blood flow) occurring secondary to heat stress-induced increases in arginine vasopressin concentration (2, 51), renal sympathetic nerve activity (21, 24), and/or hyperthermia itself (38). These changes have not been shown to be caused by reductions in perfusion pressure (26, 32) or minor dehydration (~1% loss of body weight) (19). A different, but potentially related, mechanism underlying our findings is arteriovenous shunting of blood flow within the kidney, which has been

previously suggested to occur during periods without changes in renal vascular resistance (35), and has been reported to occur in normal (3) and diabetic (42) kidneys. Doppler ultrasound would not detect if either of these changes in intrarenal blood flow occurred, because they would be downstream of our two locations of measurement. However, if intrarenal blood flow redistribution or arteriovenous shunting occurred, PAH clearance would indicate a decrease in renal blood flow in excess of any reductions in blood pressure (i.e., increased renal vascular resistance), as shown in previous studies during passive heat stress (36, 37, 46), due to a reduced blood flow to a subset of nephrons. Thus, these potential changes in intrarenal blood flow could explain our conflicting findings with previous studies.

The literature regarding the renal vascular response to sympathetic activation during heat stress is unclear. This discrepancy is likely attributed to the mode of sympathetic activation performed (e.g., exercise, head-up tilt, cold pressor test) and/or the extent of hyperthermia incurred. For example, data from Minson et al. suggest that during mild hyperthermia (i.e., increasing core temperature ~0.4°C) increases in renal vascular resistance are exacerbated during sympathetic activation (i.e., 20 min of head-up tilt) (37). However, a re-examination of data from Smith et al. show that after increasing core temperature ~0.8°C, increases in renal vascular resistance are attenuated during a sympathetic stimulus (i.e., treadmill walking) (52). Moderate hyperthermia slightly decreases mean arterial pressure and attenuates the increased blood pressure response to the cold pressor test (10). Furthermore, our study extended previous findings from our laboratory in which the increase in systemic, forearm and cutaneous vascular resistance to face cooling was attenuated during heat stress (49), adding that there is an attenuated increase in renal vascular resistance during the cold pressor test during moderate hyperthermia. The relative intensity of sympathetic stimulation evoked by the cold pressor test is not changed with heat stress (10). Therefore, it is likely that increases in renal vascular resistance during the cold pressor test were attenuated during heat stress because of a reduced vasoconstrictor responsiveness to a given level of sympathetic outflow. Similar findings have been observed in non-renal vascular beds (50). In these vasculatures, the attenuated vasoconstrictor responsiveness to sympathetic stimulation during passive heat stress is likely caused by increases in local vasodilators, such as nitric oxide, that evoke a sympatholytic effect (50). It is plausible that this same mechanism is involved in the renal vasculature given data demonstrating that elevations in nitric oxide attenuate the magnitude of angiotensin II mediated renal vasoconstriction (54). However, direct evidence is warranted.

Renal hemodynamics during cooling recovery and sympathetic activation

Rapid cooling (e.g., cold water immersion) is often employed during heat stress as a treatment to reduce the risk of a heat-related illness. However, the effect of cooling recovery on renal vascular control has not been previously

explored. During normothermia, cooling the skin, without a change in core (oral) temperature increases renal vascular resistance (56). In contrast, data in rats indicate that renal sympathetic nerve activity does not change during acute cold stress, but is reduced during hypothermia (i.e., when core temperature becomes reduced) (23). In the present study, cooling recovery was performed following passive heating and hypothermia was not the end outcome. Interestingly, we noted a period when core temperature remained elevated (by ~1.2°C) but skin temperature returned to normothermic baseline. During this period, blood velocity in the renal artery returned to normothermic baseline, yet remained unchanged in the segmental artery. Furthermore, renal vascular resistance remained unchanged from normothermic baseline in both arteries. Thus, it appears that upon the recovery of skin temperature, renal hemodynamics were restored to normothermic baseline because of the restoration of blood pressure. Notably however, it remains unknown if increases in renal vascular resistance to sympathetic activation are also restored with the recovery of skin temperature despite elevations in core temperature, as this was not examined in the present study.

Cooling recovery continued until participants reached a core temperature of 0.2 ± 0.2°C above normothermic baseline. At this point, mean arterial pressure was elevated above normothermic baseline. Renal vascular resistance in the renal artery was higher at the end of cooling recovery compared to normothermic baseline, but this differential response was not detected in the segmental artery. We believe the differential findings between the renal and segmental arteries were likely due to the slightly greater variations in mean arterial pressure and segmental artery blood velocity in this group and measurement location and were not necessarily due to physiological reasons. At the end of cooling recovery, it is likely that increased vascular resistance in the renal artery is due to higher levels of sympathetic nerve activity and/or increased concentrations of circulating vasoactive hormones (e.g., catecholamines). Cooling recovery restored the increases in mean arterial pressure and renal vascular resistance to the cold pressor test that were attenuated with passive heating. However, it is worth noting that the absolute values for both were higher in cooling recovery, as demonstrated by a higher mean arterial pressure and renal vascular resistance a cooling recovery baseline. Whether these findings are favorable for renal health is unclear. The risks of an elevated renal vascular resistance following cooling recovery are not known, but increased renal vascular resistance has been investigated as a modulator of clinical acute kidney injury (5). Thus, the importance of higher absolute values of renal vascular resistance during an acute sympathoexcitatory challenge following cooling recovery on renal health are not known, but warrant further investigation. Additionally, it remains unknown if a more aggressive cooling strategy (e.g., cold water immersion) following heat stress, which more rapidly reduces core and skin temperatures, has implications on renal vascular control.

The mechanisms underlying the restoration of the renal vasoconstrictor response to the cold pressor test during cooling recovery are not clear. It is possible that despite potential elevations in sympathetic nerve activity with cooling recovery alone (see above), the magnitude of the increase in sympathetic activation during the cold pressor test was unaffected by cooling recovery. If this were the case, it is possible that with cooling recovery the effective physiological state during sympathetic activation was functionally similar to that occurring during normothermia, suggesting that increases in local vasodilator substances (e.g., nitric oxide, ATP, etc.) had abated by this time. It is also possible that the sympathetic response to the cold pressor test was elevated with cooling recovery, but the renal vasoconstrictor response in the kidneys was offset by prevailing elevations in vasodilator substances. In theory, it is also possible that an attenuated sympathetic response to the cold pressor test during cooling recovery was offset by increases in circulating vasoconstrictors (e.g., norepinephrine) that may have been released due to cooling induced reductions in skin temperature (28). However, we believe an attenuated sympathetic response was unlikely, given that data from non-human animals indicate that reductions in renal sympathetic nerve activity are not observed until hypothermia develops (i.e., a fall in core temperature below normothermic levels) (55). Clearly, important insights could be gained by measurement of sympathetic nerve activity, particularly that of the renal sympathetic nerves. Unfortunately, in the present study we were unable to directly measure any indication of sympathetic nerve activity (e.g., muscle sympathetic nerve activity). Therefore, the mechanism(s) by which the increase in renal vascular resistance was restored to normothermic levels during cooling recovery remain largely unknown.

Renal and segmental artery response to the cold pressor test

To our knowledge, this is the first study that directly compared the renal and segmental artery responses to combined thermal and sympathetic stress. Interestingly we found a greater increase in renal vascular resistance to the cold pressor test in the segmental artery compared to the renal artery during normothermic baseline. However, this increased renal vascular resistance to the cold pressor test was abolished during both passive heating and cooling recovery. Thus, our data indicate that the effect of thermal stress on increases in renal vascular resistance to sympathetic activation were likely greater in the segmental artery compared to the renal artery. In this context, it may be more appropriate to index renal vascular resistance in the segmental artery in future studies combining thermal and/or sympathetic stressors.

Considerations

There are a few methodological considerations that warrant discussion. First, volumetric renal blood flow was not directly measured. Doppler ultrasound was used to measure renal blood velocity and interpreted to reflect changes in renal blood flow as has been discussed previously (47). Further, strict controls were set in place to increase the reliability of the operator-dependent ultrasound measurements. The intra-operator coefficient of variation of our sonographer was measured and reported to overcome limitations with interpreting measurements from Doppler ultrasound. For instance, the ~16% decrease in renal artery blood velocity at the end of passive heating was well outside the 3.9 ± 0.8% CV of the sonographer, thus, we are confident that we are measuring true physiological changes in blood velocity. Second, renal blood velocity was measured in the same segmental artery within a given participant. Thus, it is not known if measurements in the segmental artery reflect changes occurring in all segmental arteries within the kidney. Ideally, measurements in all segmental arteries would have been taken, but this is not feasible during a 2 min cold pressor test due to the controls utilized (see methods), the complexity of the technique, and the relatively limited time window within which we were able to take measurements. Third, due to the relatively short measurement windows during the cold pressor test and to ensure that participants did not hold their breath too long (which could have confounded our results), blood velocity measurements in the renal and segmental arteries were averaged across three cardiac cycles as has been done previously (12, 14, 39, 40, 43, 47). Unfortunately, therefore, we cannot be 100% certain that these three cardiac cycles are perfectly reflective of sustained changes in renal blood velocity. Fourth, we did not directly measure any indices of sympathetic activation (e.g., muscle sympathetic nerve activity), and measuring renal sympathetic nerve activity in humans would require invasive techniques. Thus, it is possible that magnitude of sympathetic stimulus evoked by the cold pressor test was not the same between normothermic baseline, passive heating, and cooling recovery despite the ~30 min between each test. However, the pressor response to the cold pressor test has been previously shown to have relatively low within subject variability when the test is repeated on the same day (17). Importantly, the magnitude of muscle sympathetic nerve activity response to the cold pressor test following passive heating is the same as normothermic baseline when completed on the same day (10).

Perspectives and Significance

There is considerable interest in understanding the pathophysiology of acute kidney injury, as the severity of acute kidney injury is associated with an increased risk of mortality (9). More recently, observational studies have reported an increased incidence of acute kidney injury during heat waves and high environmental temperatures (4, 31, 34). Data from

non-human animals indicates that heat stress evokes a heterogeneous regional redistribution of blood flow within the kidneys occurring secondary to hyperthermia (38). Our data indirectly support this contention. However, an important gap in the literature is whether these reductions in local blood flow lead to a mismatch between oxygen delivery and oxygen demand within the kidneys during heat stress. Notably, previous work from our lab suggests that mitigating the extent of hyperthermia and/or dehydration induced by heat stress may be protective for the kidneys (8, 48). The present study supports this notion such that renal artery blood velocity was restored with cooling recovery and increases in renal vascular resistance following cooling recovery were not exacerbated during sympathetic activation. However, despite these similar changes during sympathetic activation, resting blood pressure and renal vascular resistance were elevated following cooling recovery. Thus, the absolute values in blood pressure and renal vascular resistance during the cold pressor test were higher, which could have implications for cardiovascular and renal health. Despite this, relatively little is known about the renal response to cooling following heat stress. Thus, it remains unknown if these same findings would occur at a time when skin temperature returns to normothermic baseline despite an elevated core temperature, or during a more aggressive cooling intervention (e.g., cold water immersion).

Conclusion

The present study demonstrates that during the cold pressor test, increases in renal vascular resistance are attenuated during passive heating, and restored to that of normothermic baseline following a cooling recovery. Moreover, these findings were present in both the renal and segmental arteries. These data indicate that cooling recovery immediately following heat stress may not exacerbate increases in renal vascular resistance during a sympathetic stimulus, despite an elevated baseline renal vascular resistance. However, in the context of renal health, the relative importance of the higher absolute renal vascular resistance during the cold pressor test remains unknown.

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Table and Figure Legends

Figure 1 – Schematic of the study protocol. Participants underwent cold pressor tests (CPT) following normothermic baseline, after passive heating to an increase in core temperature of 1.2°C, and at the end of cooling recovery to a core temperature 0.2°C above normothermic baseline. $1.2^{\#}$ represents a period during cooling recovery when skin temperature returned to normothermic baseline, yet core temperature remained elevated by ~1.2°C. Renal ultrasound measurements (indicated as transducer symbol) were taken during the CPT, and throughout the heating and cooling protocol at the core temperatures indicated in the figure. Participants were grouped by location of ultrasound measurements for the cold pressor test: distal segment of right renal artery (Renal, *n*=12) and the middle portion of the same segmental artery within a subject (Segmental, *n*=12). Additionally, ultrasound measurements were taken in all participants in the renal artery (*n*=24), and a subset of participants in the segmental artery (*n*=12) during passive heating and cooling. The schematic is not drawn to scale in terms of time elapsed, but rather, depicts measurements as a function of the change in core temperature.

Table 1 - Core temperature, mean skin temperature, and heart rate responses at normothermic baseline (change in core temperature of 0.0°C above Baseline), during passive heating (change in core temperature of 0.6°C and 1.2°C above Baseline), and cooling recovery (change in core temperature of $1.2°C^{\#}$, 0.6°C, and 0.2°C above Baseline). $^{\#}$denotes a period when mean skin temperature returned to Baseline during cooling recovery with core temperature remaining elevated by 1.2°C. Data are presented as absolute values mean (SD). Statistical analyses from post hoc Holm-Sidak's test pairwise comparisons completed following a two-way repeated measures ANOVA: ^{B}significantly different from Baseline ($P < 0.01$). *significantly different from Heating ($P < 0.01$).

Figure 2 - Renal artery (*n*=24, A-C) and segmental artery (*n*=12, D-F) measurements during passive heating and cooling recovery. Mean arterial pressure and renal blood velocity were measured, and renal vascular resistance was calculated at

Baseline (Δ core temperature from Baseline of 0.0°C), during passive heating (Δ core temperature from Baseline of 0.6°C and 1.2°C), and during passive cooling recovery (Δ core temperature from Baseline of 1.2°C, 0.6°C, and 0.2°C). [#]denotes a period when mean skin temperature returned to Baseline during cooling recovery with core temperature remaining elevated by 1.2°C. Data are presented as the change (Δ) from Baseline as mean (SD). Statistical analyses from post hoc Holm-Sidak's test pairwise comparisons completed following a two-way repeated measures ANOVA: [B]significantly different from Δ0.0°C ($P < 0.05$). [*]significantly different from Δ1.2°C ($P < 0.01$). [†]significantly different from Δ1.2°C[#], ($P < 0.04$).

Figure 3 - Renal artery (A-C) and segmental artery (D-F) measurements for the 2 min cold pressor test (CPT) immediately before (Pre), 1 min during (1 min), at the end of 2 min (end), 1 min Post-CPT, and 5 min post-CPT. Conditions were at normothermic baseline (Normothermia, n=12), immediately following passive heating to 1.2°C above normothermic core temperature (Heated, n=12), and immediately following passive cooling recovery to 0.2°C above normothermic core temperature (Cooling Recovery, n=10). Data are presented as the change (Δ) from Pre-CPT as mean (SD). Statistical analyses from post hoc Holm-Sidak's test pairwise comparisons completed following a two-way repeated measures ANOVA: [P]significantly different from Pre-CPT ($P < 0.05$).[†]significantly different from Heated, ($P < 0.04$).

Figure 4 - Comparisons between renal artery and segmental artery measurements for renal blood velocity (A) and renal vascular resistance (B) at the end of the 2 min cold pressor test (CPT) during normothermic baseline (NT, n=12), following passive heating to 1.2°C above normothermic core temperature (HT, n=12), and immediately following passive cooling recovery to 0.2°C above normothermic core temperature (CR, n=10). Data were analyzed with unpaired t-tests and are presented as the absolute change (Δ) from Pre-CPT as mean (SD). Actual P values are reported.

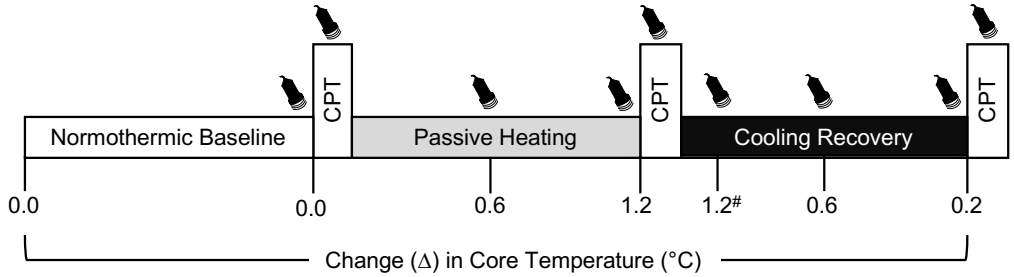
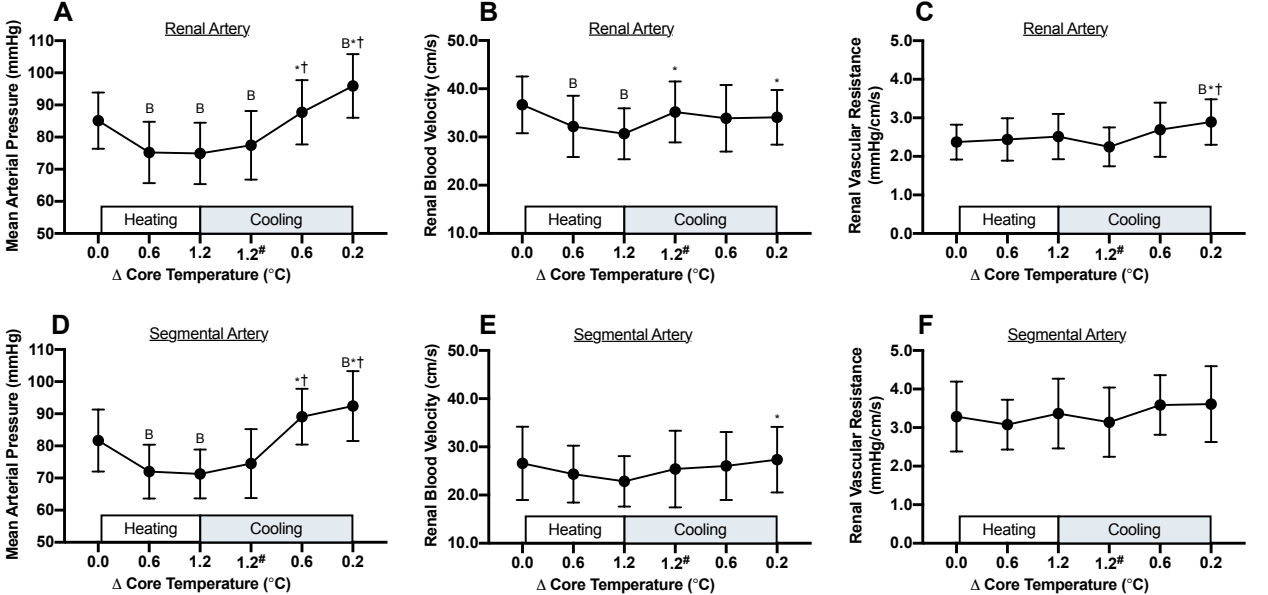
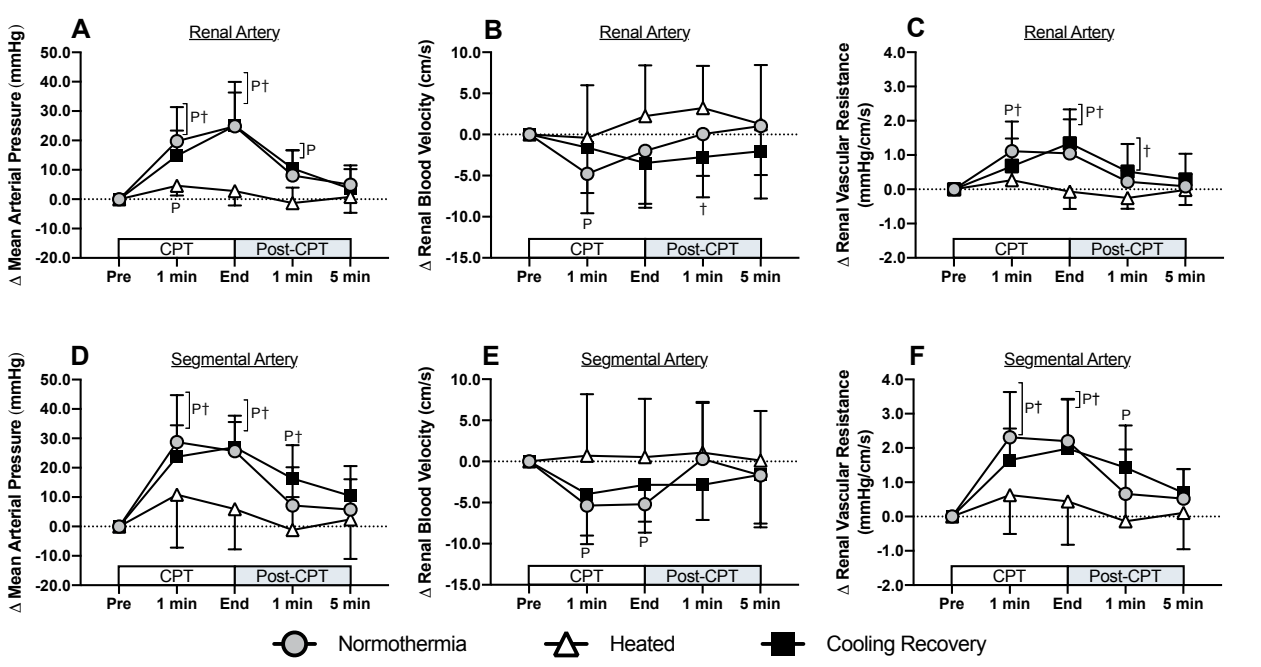
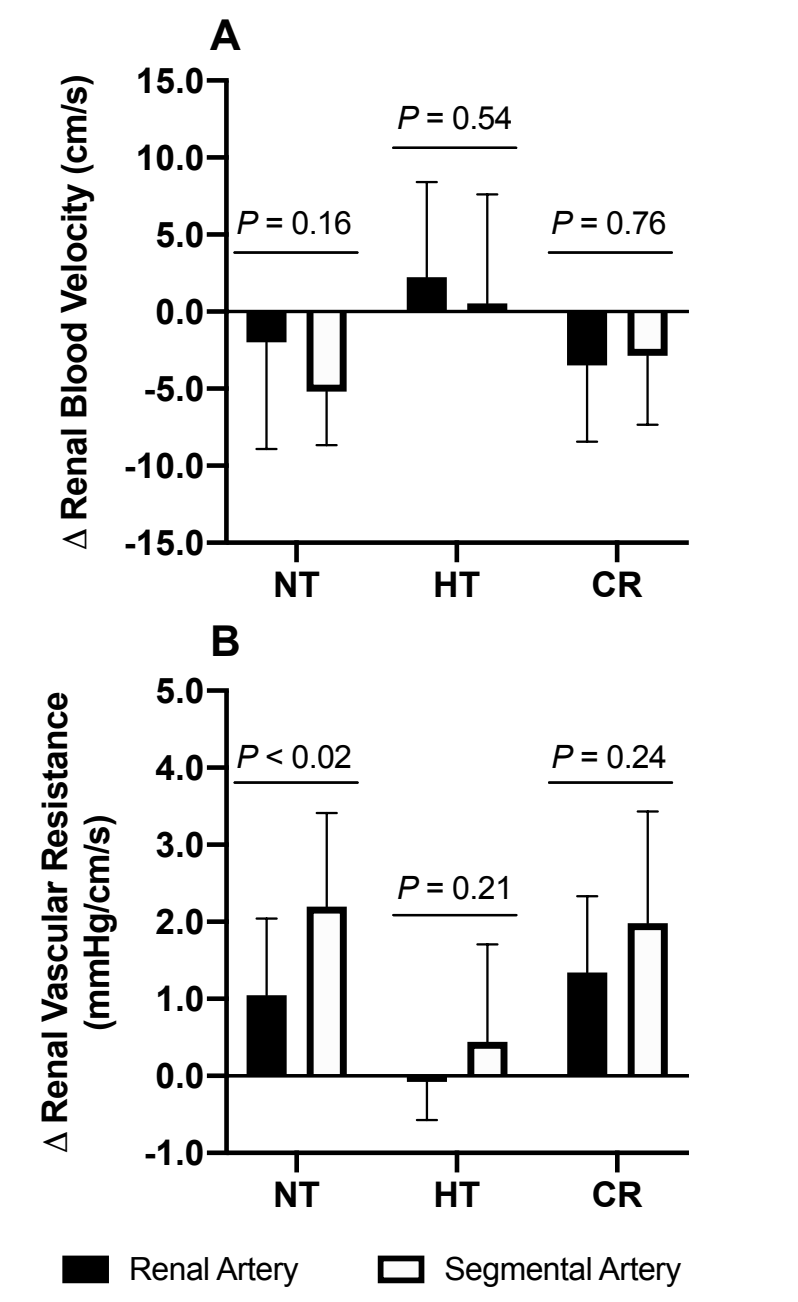

CPT
CPT
CPT
Normothermic Baseline
Passive Heating
Cooling Recovery
0.0
0.0
0.6
1.2
1.2#
0.6
0.2
Change (Δ) in Core Temperature (°C)

Table 1. *Response of physiological variables to passive heating and cooling recovery*

	Change (Δ) in Core Temperature from Baseline (°C)					
	Baseline	*Heating*		*Cooling Recovery*		
Parameter	**0.0**	**0.6**	**1.2**	**1.2**[#]	**0.6**	**0.2**
Core temperature (°C)	37.2 (0.2)	37.8 (0.3)[B*]	38.4 (0.3)[B]	38.5 (0.4)[B]	37.8 (0.2)[B*]	37.5 (0.2)[B*]
Mean skin temperature (°C)	34.8 (0.5)	38.5 (0.4)[B*]	38.9 (0.4)[B]	34.7 (0.5)[*]	33.0 (0.9)[B*]	31.3 (1.2)[B*]
Heart rate (bpm)	58 (9)	89 (13)[B*]	102 (14)[B]	70 (12)[B*]	59 (11)[*]	56 (9)[*]


A
Renal Artery
Mean Arterial Pressure (mmHg)
110
100
90
80
70
60
50
B
B
B
*†
B*†
Heating
Cooling
0.0
0.6
1.2
1.2#
0.6
0.2
Δ Core Temperature (°C)
B
Renal Blood Velocity (cm/s)
50.0
40.0
30.0
20.0
10.0
B
B
*
*
C
Renal Vascular Resistance (mmHg/cm/s)
5.0
4.0
3.0
2.0
1.0
0.0
B*†
D
Segmental Artery
E
Segmental Artery
F
Segmental Artery


A
Renal Artery
Δ Mean Arterial Pressure (mmHg)
50.0
40.0
30.0
20.0
10.0
0.0
-10.0
-20.0
P†
P†
P
P
CPT
Post-CPT
Pre
1 min
End
1 min
5 min
B
Renal Artery
Δ Renal Blood Velocity (cm/s)
10.0
5.0
0.0
-5.0
-10.0
-15.0
P
†
C
Renal Artery
Δ Renal Vascular Resistance (mmHg/cm/s)
4.0
3.0
2.0
1.0
0.0
-1.0
-2.0
P†
P†
†
D
Segmental Artery
Δ Mean Arterial Pressure (mmHg)
P†
P†
P†
E
Segmental Artery
Δ Renal Blood Velocity (cm/s)
P
P
F
Segmental Artery
Δ Renal Vascular Resistance (mmHg/cm/s)
P†
P†
P
Normothermia
Heated
Cooling Recovery


A
15.0
10.0
5.0
0.0
-5.0
-10.0
-15.0
Δ Renal Blood Velocity (cm/s)
P = 0.16
P = 0.54
P = 0.76
NT
HT
CR
B
5.0
4.0
3.0
2.0
1.0
0.0
-1.0
Δ Renal Vascular Resistance (mmHg/cm/s)
P < 0.02
P = 0.21
P = 0.24
NT
HT
CR
Renal Artery
Segmental Artery